

Effect of Clinical and Subclinical Hypothyroidism on some lipid values among Sudanese females

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Dedication

To

The soul of my great father.

My kind mother for her encourage.

*My dear husband for unlimited
support.*

My lovely kids, (Mawada & Maaza).

My brothers and sisters.

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I would like to express my sincere thanks and gratitude to my supervisor **Dr. Nabiela Musa El Bagir**, for her great efforts in directing my efforts to bring out the best we can.

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Abstract

This study composed of two parts, the first one a survey and the other one is experiment to estimate some lipids values among hypothyroidism and sub clinical hypothyroid females compared to euthyroid group. A survey was carried out for records of thyroid function test from the Radio Immuno Assay (RIA) laboratories in Sudan Atomic Energy Commission (SAEC). This was during the period from 1994-1996; the purpose was to explore the ratio of males to females, mean of their ages and the prevalence of hypothyroid function among them. About (4268) subjects visited the lab for thyroid function test (TFT), (3730) of them were found to be females while males were only about (538). Mean of their ages ranging between 30-50 years.

For the experiment part, one hundred and twenty females were selected to participate in this study, were divided into three equal groups as hypothyroidism, sub clinical hypothyroidism cases and a third group of euthyroid individuals was used as control. The levels of some serum lipids were measured (cholesterol, TG, LDL and HDL). The effect of thyroid hypo function on lipid was as follows:

1/ the hypothyroidism group showed highly significant levels of serum cholesterol, LDL –cholesterol and TG.

2/ Serum cholesterol and LDL –cholesterol levels in group with sub clinical hypothyroidism slightly elevated, in comparison with euthyroid group that represented normal values of these fractions.

In the present work a clear effect of the thyroid hormones levels was observed. The serum lipids values were influenced this was showed ($p<0.0$) as significant increase of the total cholesterol and LDL-cholesterol values in hypothyroid females.

بسم الله الرحمن الرحيم
ملخص الدراسة

hypothyroidism)

(sub clinical hypothyroidism) ((euothyroid)

(RIA)

1996 - 1994

(SAEC)

(3730)

(4268)

50 - 20

(538)

(cholesterol, TG,)

LDL and HDL

:

-1

cholesterol, LDL- cholesterol and TG.
cholesterol and LDL – cholesterol

-2

Cholesterol and LDL-

cholesterol

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Introduction

The endocrine glands and the nervous system constitute the control system in the body. They are responsible for monitoring animal's internal and external environments and making appropriate adaptive changes. The nervous system mediates its activity through nerves directly supplying the organs structures concerned, while the endocrine operates through chemical messengers known as hormones (Sukkar *et al.*, 2000, Brook and Marshal, 2001). Endocrine hypo function can be due to destruction of the gland, extra glandular disorder or defects in hormone biosynthesis. The thyroid gland is the largest organ specialized for endocrine function in the human body (Greenspan and Gardner, 2004). The thyroid hormones maintain the level of metabolism in the tissues that is optimum for their normal function. They are well known to stimulate the oxygen consumption of most of the cell in the body, helps regulate lipid and carbohydrates metabolism, modulation of gonadotropin secretion by the pituitary and maintenance of proliferative cell growth and maturation in hair. In addition, thyroid hormones stimulate both sodium pump and glycolytic pathway leading to calorigenesis and oxidative phosphorylation in tissues such as liver, kidney, and muscle (Ganog, 2001).

Hypothyroidism is a condition in which the thyroid gland does not make sufficient thyroid hormone to meet the body's requirements. In addition to the hypercholesterolemia and elevated low density lipoproteins (LDL) levels frequently observed in hypothyroidism, deficiency of thyroid hormones exert profound effects on triglycerides transport, often leading to hypertriglyceridemia. Availability of adequate thyroid hormone is essential for normal activity of lipoprotein lipase (LPL) (Williams, 1997).

Radio Immuno Assay laboratory (RIA) of Sudan Atomic Energy Commission (SAEC) is the first laboratory that conducted the routine investigations of thyroid and thyroid-related hormones since 1985; most if not all of subjects were referred to this laboratory so it will be helpful to analyze the records of the thyroid and thyroid-related hormones to monitor the prevalence of different thyroid disorders among the Sudanese population whether males or females. The first part in this study is statistical survey for the records of thyroid and thyroid-related hormones from 1994 to 1996. These records were analyzed statistically using Excel Software of Microsoft. As most of the referred subjects to the endocrines laboratories are females the second part of this study was to evaluate the lipid profiles (total cholesterol, triglycerides, low -density lipoproteins-cholesterol, and high –density lipoproteins- cholesterol) in females with hypothyroidism (have low T_4 and high TSH) and sub clinical hypothyroidism (have normal T_4 and T_3 but have high TSH) (Hardman and Limbird, 2001) in comparison to euthyroid controls (have normal T_4 , T_3 and TSH).

Objectives

1. To analyze results of hypo function of thyroid gland for subjects referred to RIA laboratory of SAEC (1994-1996) by using micro soft excel.
2. To estimate the levels of lipid profiles from blood samples of Sudanese females that are either suffering from clinical or sub clinical hypothyroid function.
3. To compare results obtained from objective 2 with results from euthyroid Sudanese females.
4. To detect the effect of clinical and sub clinical thyroid hypofunction on the lipid metabolism.

CHAPTER ONE

Literature Review

CHAPTER ONE

Literature Review

1.1 The thyroid gland

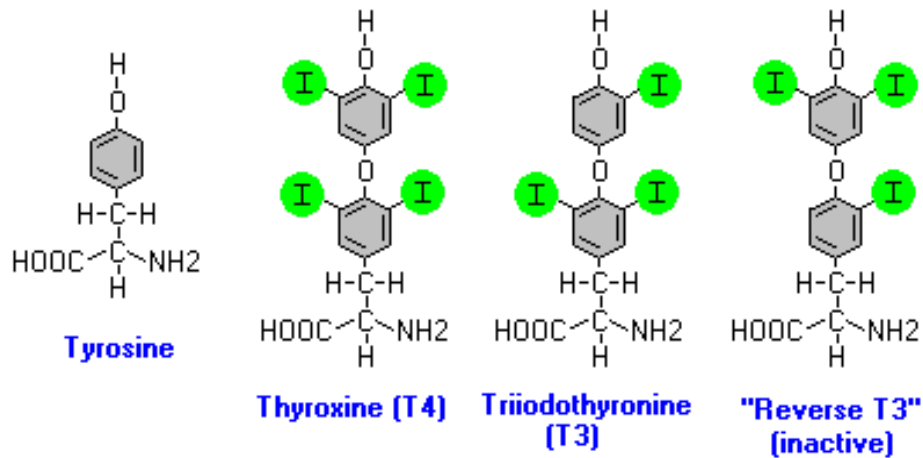
The adult human gland weight 10-20g, and is usually smaller in regions of the world in which supplies of dietary iodine are abundant. It is nearly always asymmetrical, with the right lobe often twice the size of the left one. The thyroid usually larger in women than in men and it enlarge during puberty, during lactation and in the latter part of menstrual cycle. Seasonal changes have also been reported between summer and winter, decrease in thyroid mass frequently occurs in winter. The gland is enclosed by two connective-tissue capsules. The functional unit of the gland is the thyroid follicle or acinus. This consists of cuboidal epithelial (follicular) cells arranged as roughly spheroidal sacs, the lumen of which contains colloid. The latter is composed almost entirely of the iodinated glycoprotein called thyroglobulin (Brook and Marshal, 2001). The gland has a rich blood supply and the highest rate of blood flow per gram of tissue compared to any other organ. The amount is greatly increased when the gland is overactive. The sympathetic nerves supplying the gland have no direct effect on the secretory processes (Bell *et al.*, 1972).

1.1.2 Thyroid hormones:

Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The two principal thyroid hormones are : Thyroxine, which known affectionately as T₄ or L-3, 5, 3', 5' tetraiodothyronine.

Triiodothyronine that is known as T₃ or L-3, 5, 3'-triiodothyronine. As shown in the following diagram, the thyroid hormones are basically two tyrosine residues linked together with the critical addition of iodine at three or four positions on the aromatic rings. The number and position of the iodine is important. Several other iodinated molecules are generated

that have little or no biological activity; so called "reverse T_3 " (3, 3', 5'- T_3) is such an example.



A large majority of the thyroid hormone secreted from the thyroid gland is T_4 , but T_3 is the considerably more active hormone. Although some T_3 is also secreted, the bulk of the T_3 is derived by deiodination of T_4 in peripheral tissues, especially liver and kidney. Deiodination of T_4 also yields reverse T_3 , a molecule with no known metabolic activity. T_3 and T_4 are synthesized in the colloid by iodination and condensation of tyrosine molecules bound in peptide linkage in thyroglobulin (glycoprotein contains 10% carbohydrate and 123 tyrosine residues). Thyroglobulin is synthesized in the thyroid gland cells and secreted into the colloid by exocytosis of granules that also contain thyroid peroxidase. The thyroid cells thus have three functions: they collect and transport iodine; they synthesize thyroglobulin and secrete it into the colloid and they remove the thyroid hormones from thyroglobulin and secrete them into the circulation. Thyroid hormones are poorly soluble in water, and more than 99% of the T_3 and T_4 circulating in blood are bound to carrier proteins. The principle carrier of thyroid hormones is thyroxine-binding globulin, a glycoprotein synthesized in the liver. Two other carriers of

import are transthyrin and albumin. Carrier proteins allow maintenance of a stable pool of thyroid hormones from which the active, free hormones are released for uptake by target cells. (Ganong, 2001).

1.1.3 Relation of Iodine to thyroid function

Iodine forms an integral part of the thyroxine and tri-iodothyronine molecules. Iodine is taken in food and in drinking water. A minimum daily intake of about 100-150 μg (in the form of potassium or sodium iodide) is required for normal thyroid function. The iodide is easily absorbed in the intestine to circulate in the plasma in a concentration of about 0.3 μg /100 ml; 30-50% of iodide circulating in the blood is taken up by the thyroid, while the rest is lost in the urine. The thyroid can therefore remove iodide from the blood against a concentration gradient ranging from about 40 times in a normal gland to several hundred times in an over active gland. This selective uptake of iodide by the thyroid gland, or iodide trapping, can be achieved only by active transport (iodide pump). The trapped iodide ions are oxidized into iodine in thyroid cell by means of peroxidase enzyme. The oxidized form is capable of combining with tyrosine (Sukkar *et al.*, 2000).

Adult hypothyroidism and cretinism may occur in more severe iodine deficiency. In some areas of the world, simple or non toxic goiter is prevalent because dietary iodine is not sufficient (Hardman and Limbird, 2001).

1.1.4 Thyroid hormones action

Although the thyroid gland produces more T_4 than T_3 , the latter hormone is more potent and acts much faster than the former. T_3 enters the cell more easily than the T_4 . The latter is also transformed to T_3 in the cytoplasm. T_3 binds avidly to receptors on the cell nucleus and promotes

messenger ribonucleic acid (m RNA) and ribosomal RNA (rRNA) synthesis. In most of its actions, T_3 acts more rapidly and is three to five times more potent than T_4 . This is because it is less tightly bound to plasma proteins but binds more avidly to thyroid hormone receptors. RT_3 is inert. The nuclear receptors for thyroid hormones are considered as nuclear transcription factors. One such receptor ($TR\beta_2$) is found in the brain cells only. Other receptors are found in almost all other body tissues (Sukkar *et al.*, 2000). It is likely that all cells in the body are targets for thyroid hormones. While not strictly necessary for life, thyroid hormones have profound effects on many "big time" physiologic processes, such as development, growth and metabolism. Some of the widespread effects of thyroid hormones in the body are secondary to stimulation of O_2 consumption (calorigenic action), although the hormones also affect and regulate lipid metabolism, and increase the absorption of carbohydrates from the intestine (Hardman and Limbird, 2001). Thyroid hormones increase the dissociation of oxygen from hemoglobin by increasing red cell 2, 3-diphosphoglycerate (DPG). The variety of receptors and therefore transcription of a variety of enzymes explains the wide range of effect of thyroid hormones which are shown in table 1 (Sukkar *et al.*, 2000).

Table(1): Summary of the effects of thyroid hormones (Sukkar *et al.*, 2000).

On metabolism
Increase metabolic rate
Increased O_2 consumption

<p>Calorigenic effect</p> <p>Increased Na⁺, KATPase activity</p> <p>Fat mobilization</p> <p>Increased absorption of glucose in the small intestine</p> <p>Increased formation of LDL receptors</p>
<p>Growth and development</p> <p>Normal skeletal growth</p> <p>Mental development</p>
<p>Central nervous system</p> <p>Brain development</p> <p>Potential of catecholamines, enhanced alertness</p> <p>Decreased reflex time</p>
<p>Cardiovascular system</p> <p>Increased contractility of cardiac muscles and increased heart rate</p> <p>Potential of catecholamines through increased numbers and affinity of <i>B</i>-adrenergic receptors</p>
<p>On reproduction</p> <p>Normal gonadal function</p> <p>Milk production</p>
<p>On bone marrow</p> <p>Normal erythropoiesis</p>

1.1.5 Control of thyroid function

The activity of thyroid gland is controlled by thyroid –stimulating hormone (TSH) from the anterior pituitary gland: the secretion of this is controlled by thyrotrophin releasing hormone (TRH) from the hypothalamus. Thyroid hormones (T₄ and T₃) suppress (TSH) secretion negative feedback (Brook and Marshal, 2001). TSH influences the

thyroid functions in different ways. It increases the vascularity of the gland to facilitate more efficient removal of the hormones from the gland. TSH stimulates the steps involved in the synthesis and release of thyroid hormones, from the process of iodide trapping to the activation of proteases which liberate thyroid hormones from their attachment to the thyroglobulin molecule into the colloid (Sukkar *et al.*, 2000).

1.1.6 Abnormalities in thyroid function

All thyroid disturbances are more common in females than in males (Sukkar *et al.*, 2000). Disorders of the thyroid are common. They consist of two general presentations: changes in the size or shape of the gland or changes in secretion of hormones from the gland (Hardman and Limbird, 2001). Goiter refers to any enlargement of thyroid gland, with or without disturbance of thyroid function (Sukkar *et al.*, 2000). Hypothyroidism is a clinical syndrome resulting from a deficiency of thyroid hormones, which in turn results in a generalized slowing down of metabolic processes (Greenspan and Gardner, 2004). Sub clinical hypothyroidism is an asymptomatic state characterized by elevated serum TSH concentration while serum T_4 and T_3 concentrations remain within the normal range (Hardman and Limbird, 2001). When the deficiency is congenital is referred to as cretinism. When deficiency of thyroid hormone is not congenital, the condition is referred to as myxoedema. Hyperthyroidism, which is characterized by high blood levels of thyroid hormones, can occur at an early age or it may occur later in life (Sukkar *et al.*, 2000). The term sub clinical hyperthyroidism is defined as few if any symptoms with a low serum TSH and normal concentrations of T_4 and T_3 (Hardman and Limbird, 2001).

1.2.1 Hypothyroidism

Hypothyroidism is a condition in which the thyroid gland does not make sufficient thyroid hormone to meet the body's requirements. Hypothyroidism is often referred to as an "under active thyroid gland". Caused in almost all cases by autoimmune disease, the result is that the immune system goes awry and begins gradually to destroy the thyroid gland. This process is not usually associated with thyroid gland discomfort but may cause the gland to enlarge or shrink in size.

Another important cause of hypothyroidism is the treatment of hyperthyroidism (an overactive thyroid gland), which often causes the thyroid gland to fail after a numbers of years. (Brook and Marshal, 2001).

1.2.2 Etiology and incidence of hypothyroidism

Hypothyroidism can be classified as (1) primary (thyroid failure), (2) secondary (to pituitary TSH deficit), or (3) tertiary (due to hypothalamic deficiency of TRH) or may be due to (4) peripheral resistance to the action of thyroid hormones, (Table 2). The incidence of various causes of hypothyroidism will vary depending on geographic and environmental factors such as dietary iodine and goitrogen intake, the genetic character, and the age distribution of the population (pediatric or adult) (Greenspan and Gardner, 2004).

Table (2): Etiology of Hypothyroidism: (Greenspan and Gardner,

<p>Primary:</p> <ol style="list-style-type: none">1. Hashimoto's thyroiditis:<ol style="list-style-type: none">a. With goiter.b. "Idiopathic" thyroid atrophy, presumably end- stage autoimmune thyroid disease, following either Hashimoto's thyroiditis or Grave's disease.c. Neonatal hypothyroidism due to placental transmission of TSH- R blocking antibodies.2. radioactive iodine therapy for Grave's disease3. Subtotal thyroidectomy for Grave's disease or nodular goiter.4. Excessive iodine intake (Kelp, radio contrast dyes).5. Sub acute thyroiditis.6. Iodine deficiency.
<p>Secondary:</p> <p>Hypopituitarism due to pituitary adenoma, pituitary ablative therapy, or pituitary destruction.</p>
<p>Tertiary:</p> <p>Hypothalamic dysfunction (rare)</p>
<p>Peripheral resistance to the action of thyroid hormone.</p>

2004).

1.2.3 Symptoms of hypothyroidism

The clinical signs and symptoms of hypothyroidism which are show in table 3 are manifest when the disease is fully developed. But even in the earliest (sub clinical stage), one or more of these findings may occur (Hardman and Limbird, 2001).

Table(3): The symptoms of hypothyroidism (Hardman and Limbird, 2001).

Signs and Symptoms of Hypothyroidism
Weakness, lethargy, fatigue: Dry skin Coarse hair Cold intolerance Constipation Weight gain Muscle cramps Edema of eyelids, face, legs (nonpitting) Hoarseness Hearing loss Menorrhagia Slowing of return phase of reflexes (e.g., knee jerk) Bradycardia

1.3 Sub clinical Hypothyroidism

Sub clinical hypothyroidism is defined as an asymptomatic state characterized by normal serum concentration of free thyroxine and elevated serum concentration of thyroid stimulating hormone (TSH) (Helfand and Rdfern, 1998). This biochemical state has been given a variety of other names, including mild thyroid failure, as well as compensated, early, late, mild, minimally symptomatic, and pre-clinical hypothyroidism (Arem and Escalante, 1996).

1.3.1 Causes

Subclinical hypothyroidism is caused by the same disorders of the thyroid gland as those that cause overt hypothyroidism such as chronic autoimmune thyroiditis, treated Graves' disease, congenital, hypopituitarism, iodine deficiency, radioactive therapy & antithyroid drugs. Chief among these is chronic autoimmune thyroiditis (Hashimoto's disease), which is commonly associated with increased titers of antithyroid antibodies. (Adlen, 1998). Medications such as lithium and aminodarone, sulfonyl ureas and ethioamide can interfere with thyroid hormone production or release and secondary result in a slight elevation of (TSH) (Kek *et al.*, 2003).

1.3.2 Clinical Implications

Clinical manifestations of subclinical hypothyroidism include abnormal lipid metabolism, cardiac dysfunction, and several cross-sectional studies have suggested that it confers an elevated risk of atherosclerosis and coronary heart disease. However, neither of these associations has been confirmed by others (Imaizumi *et al.*, 2004).

1.4 The Plasma lipids

Because lipids are relatively insoluble in water, they are transported in association with proteins. The simplest complexes are those formed between unesterified, or free, fatty acids (FFA) and albumin, which serve to carry the FFA from peripheral adipocytes to other tissues. The remainders of lipids are transported in spherical lipoprotein complexes, with core regions containing hydrophobic lipids. The principal core lipids are cholesterol esters and triglycerides (Greenspan and Gardner, 2004). The proteins known as apolipoprotein or apoprotein are very important since they provide structural stability to the lipoproteins, and a number of apoproteins function as ligands in lipoprotein-receptor interactions or as cofactors in enzymatic processes that regulate lipoprotein metabolism (Hardman and Limbird, 2001). In addition to FFA, four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis. These are (1) chylomicrons; derived from intestinal absorption of triacylglycerol; (2) very low density lipoproteins (VLDL), derived from the liver for the export of triacylglycerol; (3) low density lipoproteins (LDL), representing a final stage in the catabolism of VLDL; and (4) high density lipoproteins (HDL), involved in VLDL and chylomicron metabolism and also in cholesterol transport. Triacylglycerol is predominant lipids in chylomicrons and VLDL, whereas cholesterol and phospholipids are the predominant lipids in LDL and HDL, respectively. Abnormalities of lipoproteins metabolism occur at the sites of production or utilization of lipoproteins, causing various hypo- or hyperlipoproteinemias (Murray *et al.*, 2000).

1.4.1 Cholesterol metabolism

Cholesterol is the precursor of the steroid hormones and bile acids and is an essential constituent of cell membranes. It is found only in animals. Related sterols occur in plants, but plant sterols are not normally absorbed from the gastrointestinal tract. Most of dietary cholesterol is contained in egg yolks and animal fat. Cholesterol is absorbed from the intestine and incorporated into the chylomicrons formed in the mucosa. After chylomicrons discharge their triglyceride in adipose tissues, the chylomicrons remnants bring cholesterol to the liver. The liver and other tissues also synthesize cholesterol. Some of the cholesterol in the liver is excreted in the bile, both in the free form and as bile acids. Most of the cholesterol in the liver is incorporated into VLDL, and all of it circulates in lipoprotein complexes (Ganong, 2001). Cholesterol is synthesized from acetyl-CoA via a complex pathway. Three molecules acetyl-CoA form mevalonate via the important rate-limiting reaction for pathway; catalyzed by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (Murray *et al.*, 2000). Thus, when dietary cholesterol intake is high, hepatic cholesterol synthesis is decreased, and vice versa. The plasma cholesterol level is decreased by thyroid hormones, which increase the number of LDL receptors in the liver (Ganong, 2001).

1.4.2 Low Density Lipoproteins

LDL is the primary plasma carriers of cholesterol for delivery to all tissues. The exclusive apolipoprotein of LDL is apoB-100. LDL is taken up by cells via LDL receptor-mediated endocytosis. A small component is mediated by nonreceptor clearance mechanisms (Brown and Goldstein, 1986). The uptake of 75%LDL occurs predominantly in the liver (Dietschy *et al.*, 1993).

The interaction of LDL with LDL receptors requires the presence of apoB-100. The endocytosed membrane vesicles (endosomes) fuse with lysosomes, in which the apoproteins are degraded and the cholesterol esters are hydrolyzed to yield free cholesterol. The cholesterol is then incorporated into the plasma membranes as necessary. Excess intracellular cholesterol is re-esterified by acyl-CoA-cholesterol acyltransferase (ACAT); for intracellular storage. The activity of (ACAT) is enhanced by the presence of intracellular cholesterol. Thyroxine and estrogen enhance LDL receptor gene expression, which explains the LDL-C lowering effects of these hormones (Windlerr *et al.*, 1980 and Wiseman *et al.*, 1993). The elevated LDL cholesterol levels in hypothyroidism may occur as a result of increased cholesterol synthesis and absorption (Abrams *et al.*, 1981) decreased hepatic lipase and lipoprotein lipase activities (Valdemarsson and Nilsson-Ehle, 1982), and defects in the receptor-mediated catabolism of LDL (Chait *et al.*, 1979 and Thompson *et al.*, 1981). The elevation in LDL levels, in turn, may be accompanied by increased formation of oxidized LDL, which may contribute to the enhanced risk for atherosclerosis in these individuals (Oge *et al.*, 2004).

1.4.3 High Density Lipoproteins, (HDL)

The metabolism of HDL is complex because of the multiple mechanisms by which HDL particles are modified in the plasma compartment and by which HDL particles are synthesized (Tall *et al.*, 2000). ApoA-1 synthesis is required for normal production of HDL. Mutations in the apoA-1 gene that cause HDL deficiency are variable in their clinical expression and often associated with accelerated atherogenesis (Hardman and Limbird ,2001).Conversely, over expression

of apoA-1 in transgenic mice protects against experimentally induced atherogenesis (Pulump *et al.*, 1994).

The precursor of the most of the plasma HDL is a discoidal particle containing apoA-1 and phospholipid, called pre- β 1 HDL because of its pre- β 1 electrophoretic mobility. Pre- β 1 HDL are synthesized by the liver and the intestine, and they also arise when surface phospholipid and apo A-1 of chylomicrons and VLDL are lost as the triglycerides of these lipoproteins are hydrolyzed. Phospholipid transfer protein plays an important role in the transfer of phospholipids to the HDL (Tall *et al.*, 2000). After free cholesterol is acquired by the pre- β 1 HDL, it is esterified by lecithin: cholesterol acyltransferase (LCAT).

Cholesterol-rich HDL return to the liver, where they are endocytosed. Hepatic uptake of HDL, or reverse cholesterol transport, may be mediated through an HDL-specific apoA-I receptor or through lipid-lipid interactions. HDL also acquires cholesterol by extracting it from cell surface membranes. This process has the effect of lowering the level of intracellular cholesterol, since the cholesterol stored within cells as cholesteryl esters will be mobilized to replace the cholesterol removed from the plasma membrane.

The cholesterol esters of HDL can also be transferred to VLDL and LDL through the action of the HDL-associated enzyme, cholesterol ester transfer protein (CETP). This has the added effect of allowing the excess cellular cholesterol to be returned to the liver through the LDL-receptor pathway as well as the HDL. HDL is protective lipoproteins that decrease the risk of coronary heart disease (CHD); thus, high levels of HDL are desirable. This protective effect may result from participation of HDL in reverse cholesterol transport, the process by which excess cholesterol is

acquired from cells and transferred to the liver for excretion. HDL also may inhibit oxidative modification of LDL through the action of paroxonase, an HDL-associated antioxidant protein (Hardman and Limbird, 2001). Thyroid hormones appear to stimulate virtually all aspects of lipid metabolism, including synthesis mobilization, and degradation (Williams, 1997).

1.5 Hyperlipidemia and Hypothyroidism

In addition to the hypercholesterolemia and elevated LDL levels frequently observed in hypothyroidism, deficiency of thyroid hormones exert profound effects on triglycerides transport, often leading to hypertriglyceridemia. Availability of adequate thyroid hormone is essential for normal activity of LPL. In hypothyroidism, low LPL activity appears to be reciprocally related to hypertriglyceridemia, and the abnormalities. The relationship between mild thyroid failure and reversible elevation in serum lipid levels has been widely investigated, but, the findings remain controversial. In patients with full-blown hypothyroidism, serum levels of triglycerides (TG), total cholesterol and low-density lipoproteins (LDL) cholesterol are elevated. In patients with sub clinical hypothyroidism, not surprisingly, the same changes are present but are less marked and less consistent. This pattern of lipid abnormalities, of course, is important because it is a risk factor for atherosclerotic cardiovascular disease (Adlen, 1998). Hypothyroidism can alter lipoprotein metabolism in the following ways: decrease lipoprotein lipase activity, increase hepatic production of LDL-C, decrease LDL-C receptor activity, and reduce HDL-C binding to hepatocytes. It has been suggested that patients with hypothyroidism are at increased risk for coronary artery disease. Atherogenic changes in the lipid profile, such as elevation of levels of TC, LDL-C, and TGs, have been described.

Changes in HDL-C, apolipoprotein (apo) A-I, and lipoprotein (a) (LP[a]) have been noted to vary (Diekman *et al.*, 1995).

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1. The Survey

A general survey was carried out for the records of thyroid function test to subjects visited the SAEC laboratories during 1994-1996. The study focused on the variation in sex, age and subject's status, whether they were new cases or under treatment this is related to their thyroid status. The statistical analysis was done using statistical package for social science (spss) and excel software of Microsoft.

2.2. Subjects

One hundred and twenty adult female patients their ages ranging between 30-55 years were selected from the subjects referred to the Radiation and Isotopes Center, Khartoum (RICK), Radio- immunoassay laboratory, during February, 2005 to July, 2005, suspected as cases of thyroid dysfunction. Subjects were divided into three equal groups: euthyroid as control, hypothyroidism and subclinical hypothyroid group. Subclinical hypothyroidism is a condition in when there is small elevation in thyroid stimulating hormone, yet normal circulating levels of thyroid hormones (William and William, 2004) .In the present study subjects that considered as sub clinical hypothyroid cases had serum TSH levels between (6.0-10 mIU/l) and euthyroid values for T3 and T4.

2.3. Samples and sampling techniques

Blood samples (5 ml) were collected from cubital vein after overnight fasting. The blood samples were allowed to clot at room temperature and then centrifuged at 4000 R.P.M to obtain the serum. The clear serum was taken immediately for analysis or stored at -20 C° for further use.

2.4. Laboratory methods

The levels of TSH, T4, and T3 were estimated by using Radioimmunoassay (RIA) techniques, while commercial enzymatic

methods were used for determination of total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol.

2.4.1. Radioimmunoassay (RIA)

(RIA) kits are all supplied by the Department of Isotopes, China Institute of Atomic Energy, 1994. Each Kit has specific code: IMK-437, IMK-438, and IMK-432 for T₄, T₃ and TSH respectively.

2.4.1.1. T4 kits

These kits consisted of 6 vials of lyophilized standards T4 to give different concentrations when reconstituted by 1 ml of distilled water; (0.0, 26, 52, 103, 206 and 309 nmole/l) thyroxin.

One vial of T4-I¹²⁵ tracer and T4 antibodies coupled to magnetic particles, and one vial for quality control (QC) sample.

2.4.1.2. T3 kit

Includes 6 vial of triiodothyronine standard solution in different concentrations, (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 nmol/l).

One vial contains T3-I¹²⁵ tracer, one vial Anti-T3 antibody, and one vial of (QC) sample.

2.4.1.3. Thyroid stimulating hormone (TSH) kit

Contains 7 vials of standard solutions of TSH; their concentrations as follow: A. 0.0, B. 0.23, C. 1.0, D. 3.0, E. 10.0, F.20.0 and G. 80.0 mIU/L.

One vial contain anti-TSH antibodies coupled to magnetic particles, (solid phase separation system), one vial of tracer (labeled anti TSH with I¹²⁵), one vial wash Buffer, 3 vial of QC samples A, B and C with different ranges.

Equipment

1. Adjustable micropipettes (10-200 μ l) with disposable tips.
2. Polystyrene test tubes (disposable).
3. Vortex mixer (single and multi-tubes).
4. Multidose micropipette. (Eppendorfe).25 μ l and 250 μ l, magnetic base (separator).
5. Water bath.
6. Incubator.
7. Centrifuge.
8. Gamma counter, connected with computer contains (IAEA) programme (International Atomic Energy Agency).

2.4.1.4. T4 assay procedure

Sufficient (polystyrene) test tubes were labeled in duplicates and arranged in assay rack, and then 25 μ l were pipetted into each tube of the standards, quality control sample and patient's sample. And 250 μ l anti T4 antibody were added to each tube, and mixed well, to the STD and QC and samples 250 μ l of tracer were added.

After good mixing tubes were incubated at 37° C in the incubator for 45 minutes, then the rack was placed in the magnetic base for 10 minutes, to separate the bound fraction from the free fractions by decanting the supernatant. Lastly each tube was counted in the gamma counter to evaluate the gamma emission per minutes, and binding percent was plotted vs. the concentration, to get standard calibration curve, and from the obtained curve the concentration of thyroxin in the patient's samples was evaluated. This method is bioassay method, (Radioimmunoassay), Using radio active isotope of iodine (I^{125}) which is gamma emitter.

2.4.1.5. T3 assay

Sufficient number of test tubes were labeled in duplicate and arranged in assay rack. 25 µl of standard solutions, QC samples and patients sample were added to each target tube. 250 µl of T3- I¹²⁵ tracer and 100 µl anti-T₃ antibodies were added to each tube and mixed, the tubes were incubated at 37°C for one hour and then vortex well and centrifuged to separate bound fraction, (liquid phase separation system). The supernatant was decanted and then each tube was placed in gamma counter. The principle of the assay is the same as that for T₄.

2.4.1.6. TSH assay

Sufficient test tubes were labeled and arranged in assay rack in duplicates. 100 µl of STD and QC and samples were pipetted in target tubes and 25 µl tracer (anti TSH labeled by I¹²⁵) were added to each tube and vortexed gently, and then incubated at 37°C in the incubator for one hour. 250 µl of anti TSH (antibody coupled to magnetic particles) were added to each tube and well mixed and incubated at 25°C for one hour. Then the racks were placed in the magnetic separator for 10 minutes and the supernatant was separated by decantation.

Wash Step

In this step, first the concentrated wash buffer was diluted by adding water (1:9), and then 500 µl of the diluent was added to each tube and then vortexed well and then placed again in the magnetic base and allowed to stand for 10 minutes. Then the supernatant was decanted and drained thoroughly on adsorbent paper. The wash step was repeated again. All the tubes were counted in the gamma counter, to evaluate the concentration of TSH in the patient sample.

The quantitative analysis of TSH is achieved by the above method, which is immunoradiometric method. It is non- competitive method in which the radio active compound (tracer) is TSH antibody. There are two antibodies react with the TSH in the analyte to get a sandwich complex.

Calculation of results

The results in these two methods are obtained by using computerized method in which software provided from International Atomic Energy Agency (IAEA) was used.

The count per minute of each tube was counted in the Gamma counter, to get the emission from all tubes in one minute including the patients samples, the standard curve was plotted, the concentration of T₃, T₄, TSH standards in the X axis Vs. the bound percentage (the count of each standard over the total count B/T%) in the y axis.

$$100 \times \frac{\text{Count of std/sample}}{\text{Total count}} = \text{Binding \%}$$

Then concentration of each sample was calculated from the standard curve (normal interpolation).

2.4.2. Estimation of Serum Lipids

Equipments and reagents

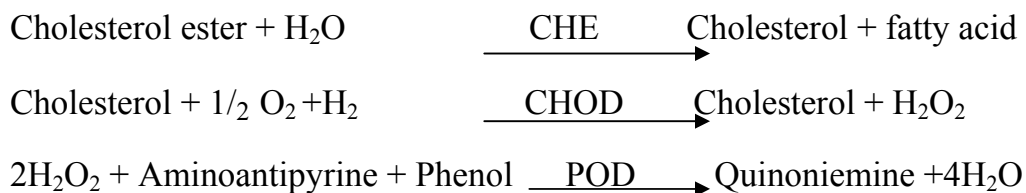
1. Polystyrene test tubes (disposable).
2. Vortex mixer (single and multi-tubes).
3. Multidose micropipette. (Eppendorfe).25µl and 250 µl,
4. Water bath.
5. Spectrophotometer (Biosystem 305- BTS), with 500 nm filter (490-510).

- All kits and reagents were supplied by Biosystem Company, for reagents and instruments. Methods and procedures are applied according to the instruction described in the kits manuals. Each Kit has specific code: 11505 (1 X 200 ml), 11648, 11579, and 11528 for Cholesterol, HDL-C, LDL-C, and TG respectively.

2.4.2.1. Estimation of total cholesterol

Principle of the method

In the presence of cholesterol esterase, the cholesterol esters in the sample are hydrolyzed to cholesterol and free fatty acids. The cholesterol produced is oxidized by Cholesterol oxidase to cholestenone and hydrogen peroxide. Hydrogen peroxide is detected by a chromogenic oxygen acceptor, phenol- ampyrone, in the presence of Peroxidase. The red quinone formed is proportional to the amount of cholesterol present in the sample. (Deeg and Ziegenhorn 1983).



Contents and composition of reagents

- 1- Reagent-1 (Buffer): PIPES PH 6.9 90 mmol/l, phenol 26 mmol/l
- 2- Reagent-2 (Enzymes): Cholesterol esterase (CHE) 300 U/ml, Cholesterol oxidase (CHOD) 300 U/L, Peroxidase (POD) 1250 U/ml, 4-aminoantipyrine (4-AP) 0.4 mmol/l, pH 7.0.
- 3- Cholesterol standard 200 mg/dl.

Procedure

- 1- The working reagent was prepared by dissolving one vial of Reagent-2 enzymes in one bottle of reagent-1 Buffer.
- 2- The working reagent was brought to room temperature.
- 3- Into labeled test tubes the following were pipetted:

	Blank (B)	Standard (STD)	Sample (S)
Cholesterol standard		10 µl	
Sample	-	-	10 µl
Working reagent	1.0ml	1.0ml	1.0ml

- 4- The tubes were incubated for 10 minutes at room temperature (16-25 C°).
- 5- The absorbance (A) of the standard and the samples were measured at 500 nm against the blank.

Calculatoins

$$\text{Cholesterol (mg/dl)} = \frac{AS}{AST} \times 200$$

AS: means absorbance of the sample.
standard

AST: means absorbance of the

2.4.2.2. Estimation of high- density lipoprotein Cholesterol(HDL-C)

Principle of the method

Very low-density lipoprotein (VLDL) and low density lipoprotein (LDL) in the sample are precipitated with phosphotungsate and magnesium ions. The supernatant contains high-density lipoproteins (HDL). The HDL-

cholesterol is then spectrophotometrically measured by means of the coupled reactions as described in total cholesterol previously.

Contents and composition of reagents

- 1- Reagent B: 50 ml, phosphotungstate 0.4 mmol/L, magnesium chloride 20 m mol/L
- 2- HDL-cholesterol standard 3 ml cholesterol 40 mg/dL
- 3- Cholesterol Kit (Biosystemcode. 11505, 11506, 11539)

Procedure

Precipitation

- 1- Into labeled test tubes the following were pipetted:

Sample	0.2 ml
Reagent B	0.5 ml

- 2- The tubes were let to stand for 10 minutes at room temperature.
- 3- Centrifuged at minimum of 4000 r.p.m for 10 minutes.
- 4- The supernatant was collected carefully.

Colorimetry

- 1- The cholesterol reagent was brought to room temperature.
- 2- Into labeled test tubes the following were pipetted:

	Blank (B)	Standard (STD)	Sample (S)
Distilled water	20 µl	-	-
Cholesterol standard		20 µl	-
Sample supernatant	-	-	20 µl
Cholesterol reagent	1.0 ml	1.0 ml.	1.0 ml

- 3- The tubes were incubated for 10 minutes at 37 C°.
- 4- The absorbance (A) of the sample and the standard were measured at 500 nm against the blank.

Calculations

$$\text{HDL-cholesterol (mg/dl)} = \frac{\text{AS}}{\text{ASt}} \times 3.5 \times 40$$

2.4.2.3. Estimation of low- density lipoprotein cholesterol (LDL-C)

Principle of the method

Low- density lipoprotein (LDL) in the sample is precipitated with poly vinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of the coupled reactions as described in total cholesterol previously.

Contents and composition of reagents

- 1- Reagent B: 10 ml polyvinyl sulphate 3 g/L, polyethylene glycol 3 g/L.
- 2- Cholesterol kit (Biosystem code 11505, 11506, 11539).

Procedure

Precipitation

- 1- Into labeled test tubes the following were pipetted:

Sample	0.2 ml
Reagent B	0.1 ml

- 2- The tubes were let to stand for 10 minutes at room temperature.

3- Centrifuged at minimum of 4000 r.p.m for 10 minutes.

4- The supernatant was collected carefully.

Colorimetry

1- The cholesterol reagent was brought to room temperature.

2- Into labeled test tubes the following were pipetted:

	Blank (B)	Standard (STD)	Sample (S)
Distilled water	20 μ l	-	-
Cholesterol standard	-	20 μ l	-
Sample supernatant	-	-	20 μ l
Cholesterol reagent	1.0 ml	1.0 ml.	1.0 ml

3- The tubes were mixed and incubated at room temperature for 30 minutes.

4- The absorbance (A) of the sample and the standard were measured at 500 nm against the blank.

Calculations

The dilution factor of the sample in the precipitation is 1.5 and the concentration of the standard is 200 mg/dL according to the following formula is;

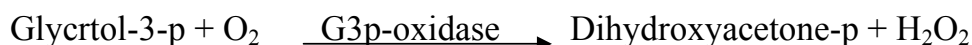
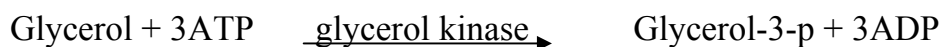
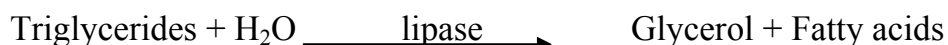
$$\text{cholesterol in the supernatant (mg/dl)} = \frac{A_s}{A_{st}} \times 1.5 \times 200$$

LDL- cholesterol = Total- cholesterol – Cholesterol in the supernatant.

2.4.2.4. Estimation of triglycerides

Principle of the method

Triglycerides in the sample originate, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry (Fossati and Prencipe, 1982).



Contents and composition of reagents

- 1- Reagent A. PIPES 45mmol/L, magnesium chloride 5mmol/L, 4-chlorophenol 6mmol/L, lipase > 100 U/ml, glycerol kinase > 1.5 U/ml, glycerol-3-phosphate oxidase > 4 U/ml, peroxidase > 0.8 U/ml, 4-aminoantipyrine 0.75mmol/L, ATP 0.9mmol/L, PH 7.5.
- 2- Triglycerides standard. Glycerol equivalent to 200 mg/dl triolein.

Procedure

- 1- The Reagent was brought to room temperature.
- 2- Into labeled test tubes the following were pipetted:

	Blank (B)	Standard (STD)	Sample (S)
Triglycerides standard	-	10 μ L	-
Sample	-	-	10 μ L
Reagent	1.0mL	1.0mL	1.0mL

- 3- The tubes were incubated for 15 minutes at room temperature.
- 4- The absorbance (A) of the standard and sample was measured at 500 nm against the blank.

Calculations

$$\text{triglycerides (mg/dL)} = \frac{AS}{AS_t} \times 200$$

2.5. Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS 10.1). Different statistical methods were used as appropriate. Mean \pm SD was determined for quantitative data and frequency for categorical variables.

Chapter Three

Results

Chapter Three

Results

3.1 The survey

3.1.1 Statistical analysis of subjects who referred to the RIA laboratory, of SAEC (1994-1996):

The Sudanese subjects who referred to the RIA laboratory of SAEC to do thyroid function test during the period from 1994 to 1996 were about 4268 ; among them 3730 females that represent 87.4% compared to 538 males represent 12.6%. 85.13% of them were new cases and 14.87% were under treatment .The percentage of hypothyroidism, subclinical hypothyroidism and euthyroid among male subjects were about 1.6, 6.1 and 75.80% respectively.

New cases females were about 85.42%, while 14.58% under treatment. Whereas, the percentage of hypothyroidism, subclinical hypothyroidism and euthyroid among female subjects were about 4.6, 6.2 and 78.3% respectively (table 5). However, almost 85% of the referred patients visited the RIA laboratory for the first time as shown in table (4).

Table (4) The percentages of Sudanese subjects visited RIA Lab, SAEC during (1994-1996).

Sex	Under treatment		New cases		total
	no	%	no	%	
Male	80	14.87	458	85.13	538
Female	544	14.58	3186	85.42	3730
Total	624	14.71	3644	85.29	4268

Fig (1) shows the type of visit to the RIA laboratory for the group under survey while fig (3) shows the results of thyroid function test for both males and females; who visited the RIA laboratory at SAEC during the period 1994 to 1996. However, fig (4), (5) and (6) represent the mean values for T4, T3 and TSH for Sudanese subjects respectively.

Table (5) The thyroid status among males and females under survey

Category	Prevalence among Sudanese males (%)	Prevalence among Sudanese females (%)
Euthyroid	67.1%	71.4%
Hypothyroid	1.7%	4.7%
Subclinical hypothyroid	6.13%	6.2%

Fig (1): The type of visit to the RIA laboratory for the subjects under survey.

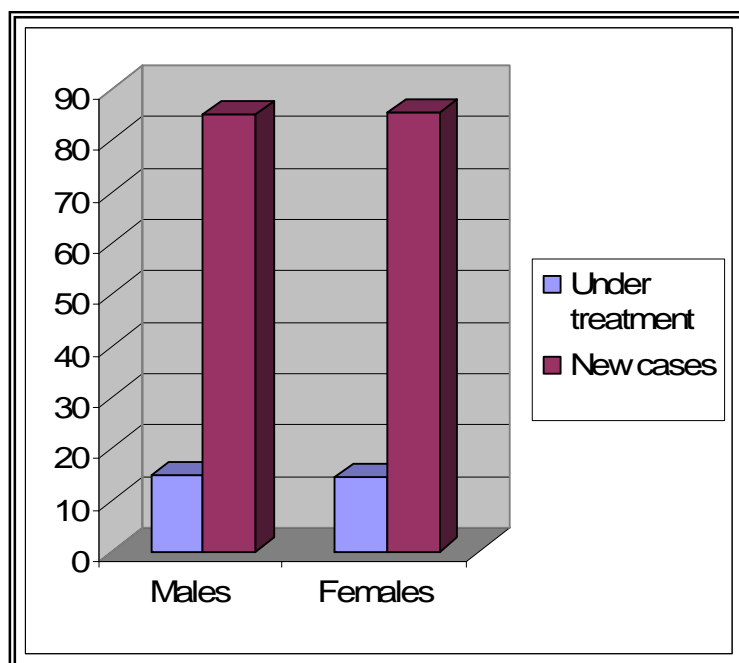


Fig (2): Mean of age in years for patients under survey.

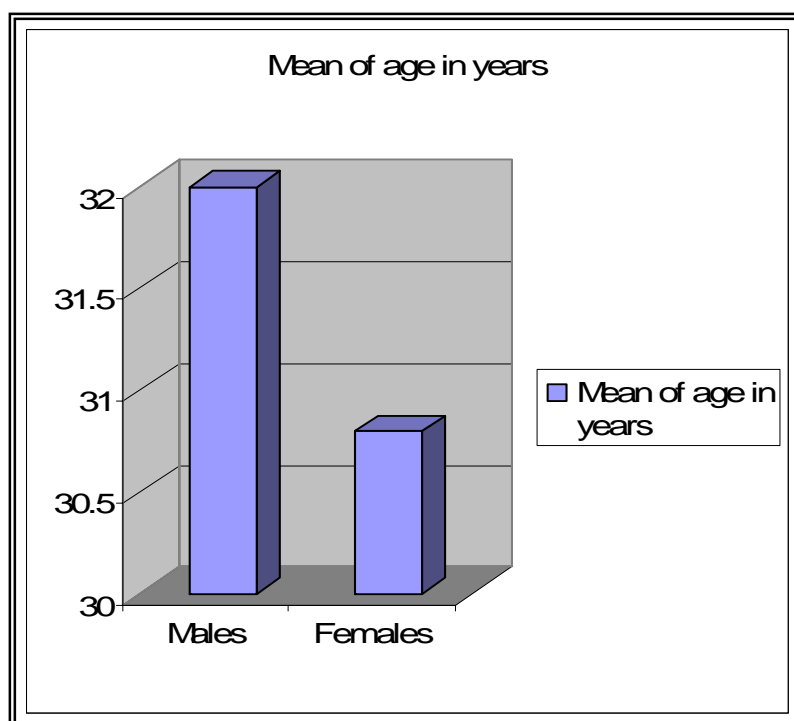


Fig (3): The prevalence of hypothyroidism (clinical and subclinical) compared to euthyroid among the Sudanese subjects (1994-1996).

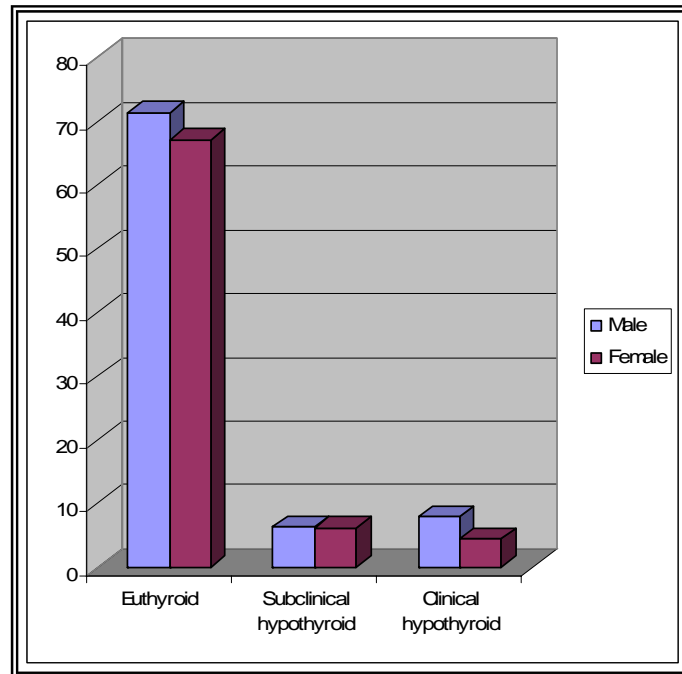


Fig (4): Mean values of T₄ (nmole/L) for euthyroid and hypothyroid (clinical and subclinical) subjects under survey.

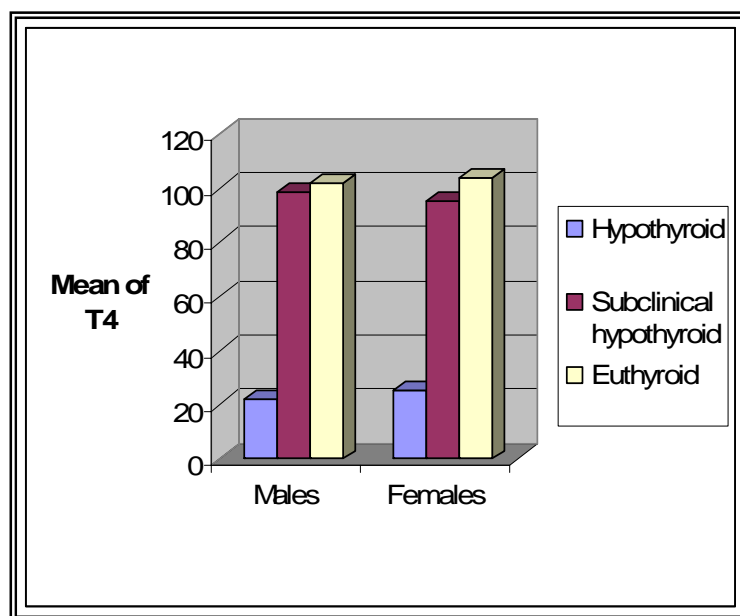


Fig (5): Mean values of T₃ (nmole/L) for euthyroid and hypothyroid (clinical and subclinical) subjects under survey.

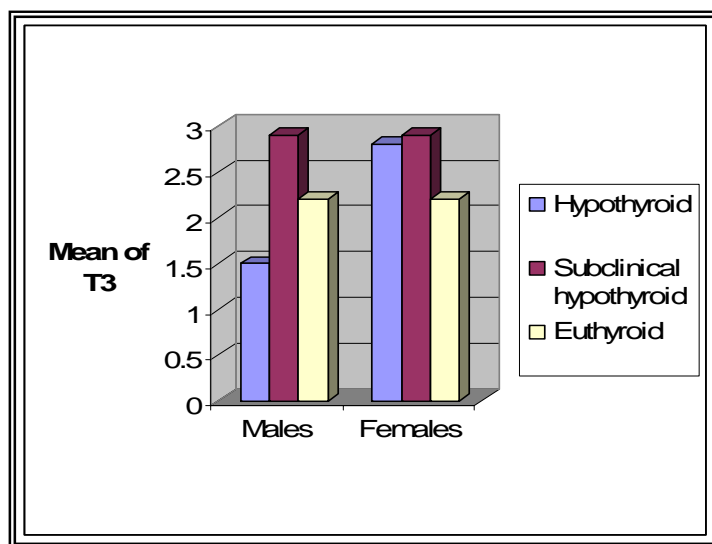
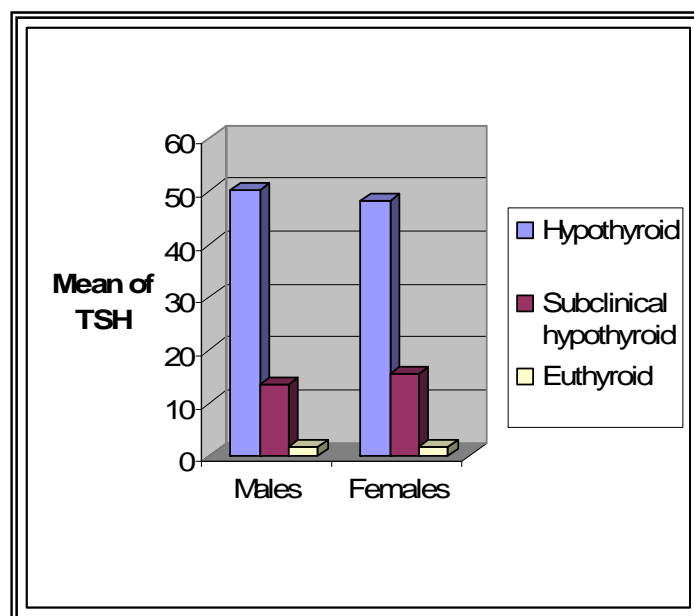


Fig (6): Mean values of TSH (mIU/ml) for euthyroid and hypothyroid (clinical and subclinical) subjects under survey.



3.2 Thyroid function test status in the study group of Sudanese females:

A total of one hundred and twenty females were selected to participate in the present study. 40 hypothyroid females, equal number fit the criteria for subclinical hypothyroidism cases, and the remaining 40 were euthyroid selected as controls. The mean of age for euthyroid about 32 years and 34 years for hypothyroid subjects and about 44 for Sub clinical hypothyroid subjects (fig 7).

The mean level of serum T_4 among euthyroid, hypothyroid and subclinical hypothyroid subjects were found to be 94.30, 18.4 and 89.7 nmole/L respectively, hypothyroid cases, showed significantly lower levels of T_4 , ($P < 0.001$), but subclinical hypothyroid subjects showed non significantly different level of T_4 compared to the control (Table 6 Fig 8 and 9).

Mean values for serum T_3 among euthyroid, hypothyroid and sub clinical hypothyroid groups were found to be 1.96, 1.19 and 1.95 nmole/L respectively (Table 6 Fig 10 and 11). The euthyroid mean level of TSH, when determined in this study, was found to be $(1.60 \pm 0.54 \text{ mIU/ml})$. Hypothyroid cases showed significantly higher levels compared to euthyroid ($P < 0.001$) with TSH mean about (49.6 mIU/ml) . Among subjects with subclinical hypothyroidism, also there was a significantly higher TSH level compared to euthyroid subjects, ($P < 0.005$) (Table 6, Fig 12 and 13).

Table (6): The levels of thyroid and thyroid stimulating hormones(Mean \pm SD).

Thyroid status	Thyroxine	T3	TSH
Normal ranges	50-150 nmole/L	0.80-3.0 nmole/L	0.40-4.0 mIU/ml
Euthyroid subjects	94.30 ^a \pm 9.97	1.97 ^a \pm 0.46	1.60 ^a \pm 0.54
Hypothyroid subjects	18.4 ^b \pm 9.3	1.19 ^a \pm 0.79	49.6 ^{b**} \pm 25.57
Subclinical hypothyroid subjects	89.7 ^a \pm 11.39	1.9 ^a \pm 0.45	9.6 \pm ^{a*} 2.69

- Mean within the same column having different small letters were significantly different.
- ** ($p < 0.001$) and* ($p < 0.005$).

Fig (7): Mean of age in years for euthyroid and hypothyroid (clinical and sub clinical) subjects under study

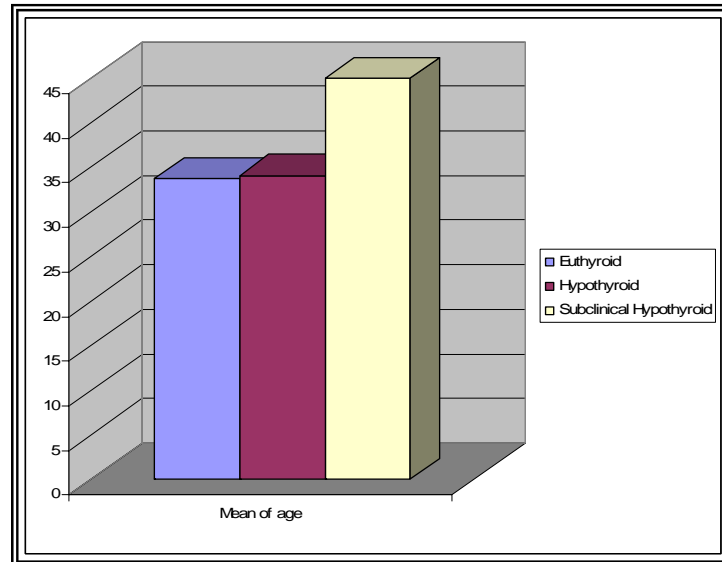


Fig (8): Mean values of T4 (nmole/L) of euthyroid hypothyroid and sub clinical hypothyroid subjects.

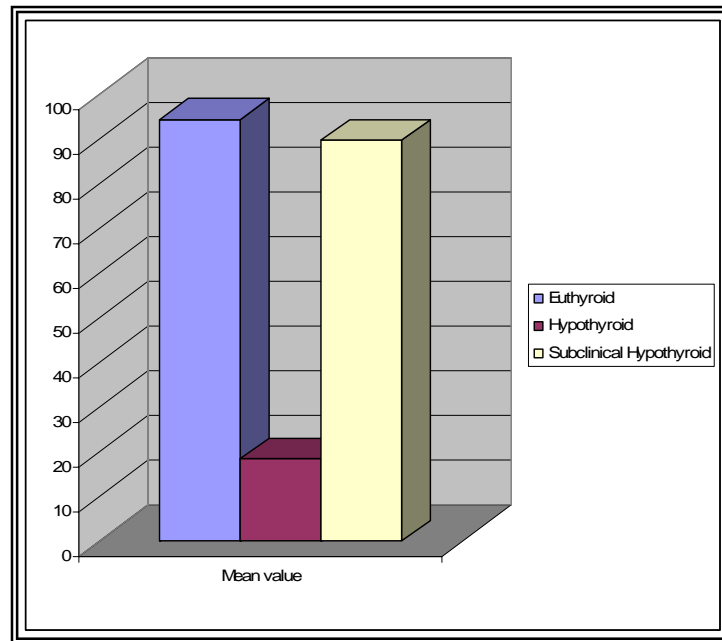


Fig (9): Prevalence of T4 levels (nmole/L) among euthyroid, hypothyroid and sub clinical hypothyroid Sudanese Females

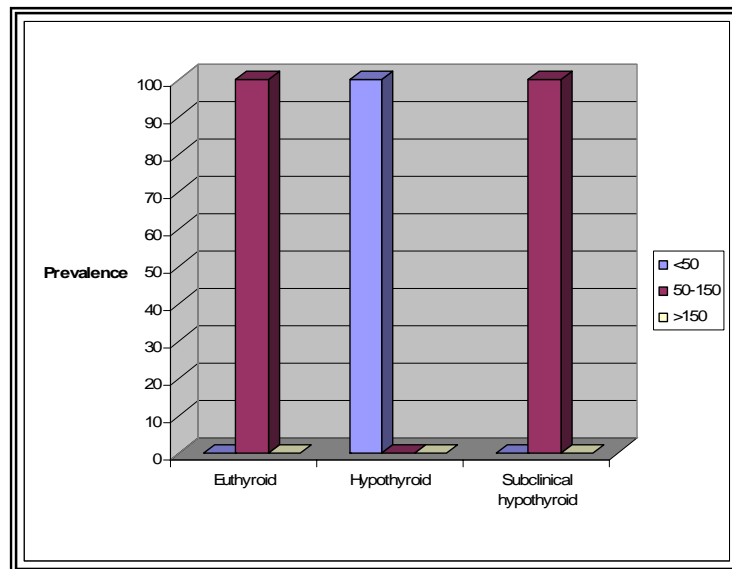


Fig (10): T3 Levels (nmole/L) of euthyroid, hypothyroid and sub clinical hypothyroid subjects.

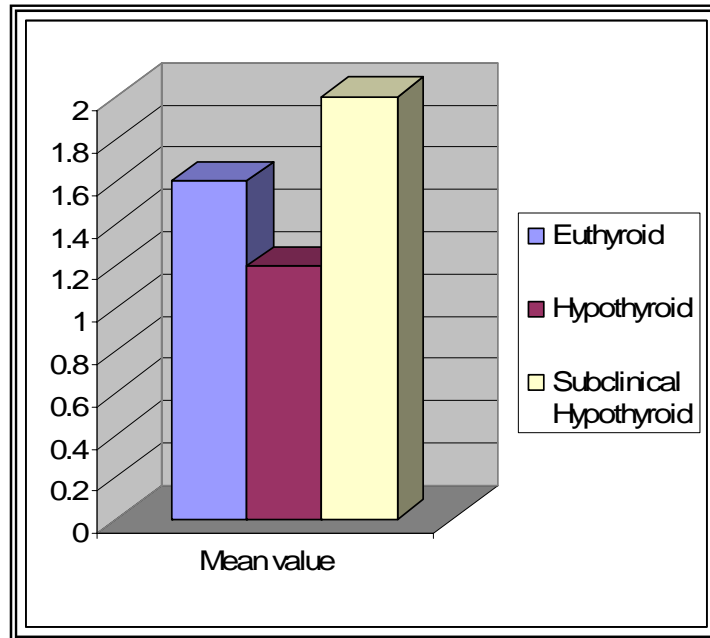


Fig (11): Prevalence of T3 levels (nmole/L) of euthyroid, hypothyroid and sub clinical hypothyroid subjects.

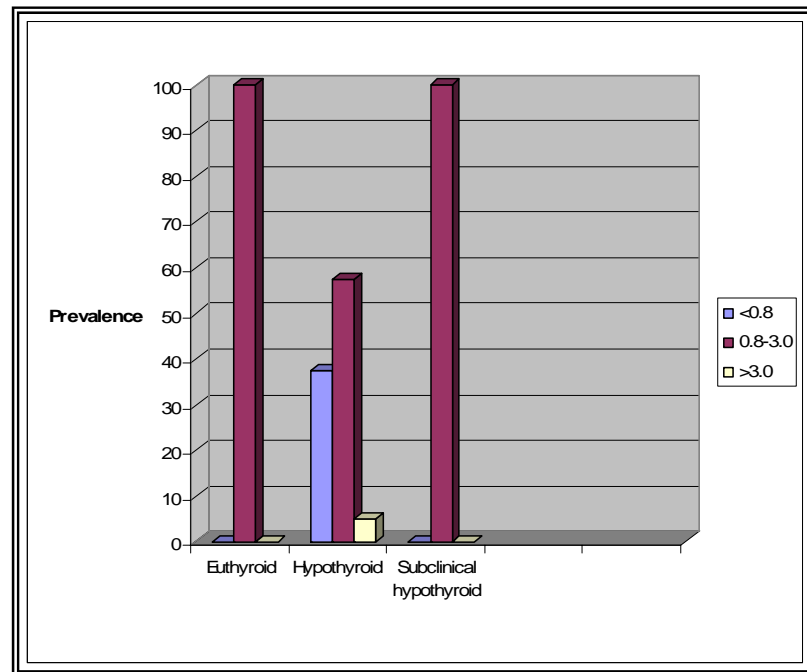


Fig (12): Mean levels of TSH (mIU/ml) of euthyroid, hypothyroid and sub clinical hypothyroid subjects.

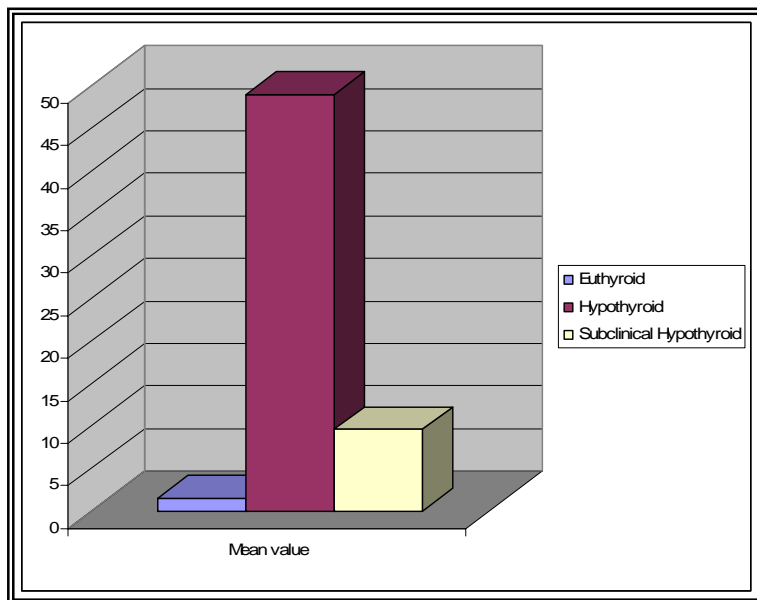
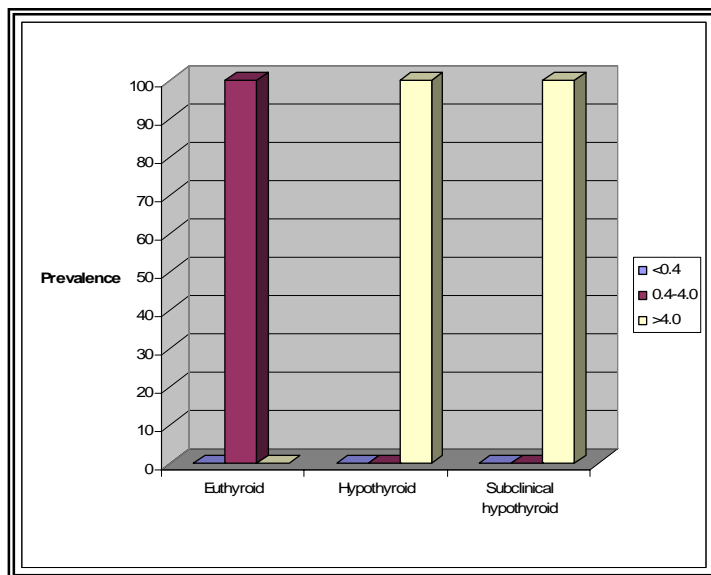


Fig (13): The prevalence of TSH levels (mIU/ml) among Sudanese females under study.



3.3 Serum Lipid profiles results

3.3.1 Triglycerides (TG) level among study groups

The mean values of triglycerides were shown in figure 14 and table 7 while the prevalence of triglycerides in the different three groups was shown in figure 15. Hypothyroidism showed significantly higher levels of TG ($P < 0.001$) compared to euthyroid subjects. Also there was statistically significant relationship between sub clinical hypothyroid cases and the euthyroid ($P < 0.001$), that sub clinical hypothyroid subjects showed high TG level.

3.3.2 Total cholesterol level among study groups

Total cholesterol levels were measured in the three groups using colorimetric method; the mean value of cholesterol was (172.68 ± 31.0 , 194.5 ± 40.51 227.6 ± 59.88) mg/dl among euthyroid, sub clinical hypothyroid and hypothyroid subjects respectively, (as shown in figures 16 and table 7) while the prevalence in the different groups was shown in figure 17. Hypothyroidism showed significantly higher levels of cholesterol ($P < 0.001$) compared to euthyroid subjects. However subjects with sub clinical hypothyroidism showed significantly high levels of cholesterol but less than of hypothyroidism ($P < 0.05$) compared to euthyroid subjects.

3.3.3 High density lipoprotein cholesterol (HDL) level among study groups

The fractions HDL- cholesterol was analyzed, results were represented in table 7 .Figure 18 shows the mean values of HDL-cholesterol. While figure 19 shows the prevalence of HDL-cholesterol in the different groups of Sudanese females. There was no significant difference reported in the levels of HDL-C ($P < 0.9$). The mean of the serum levels among the controls and the sub clinical hypothyroid subjects were (52.05 ± 11.5 , 51.3 ± 17.13 and 52.2 ± 9.58) mg/dl respectively.

3.3.4 Low density lipoproteins cholesterol (LDL) level among study groups

The fractions LDL- cholesterol was analyzed, results were represented in table 7 .Figure 20 shows the mean values of LDL-cholesterol. While figure 21 shows the prevalence of LDL-cholesterol in the different groups of Sudanese females. Hypothyroidism showed significantly higher levels of LDL- cholesterol ($P < 0.001$) compared to euthyroid subjects. When LDL-C levels were considered, also there was statistically significant relationship between sub clinical hypothyroid cases and the controls ($P < 0.05$).

Table 7 the results of triglycerides, total cholesterol, HDL-cholesterol and LDL- cholesterol expressed as mean \pm SD among the euthyroid and hypothyroid and sub clinical hypothyroid subjects.

Thyroid	TG	TC	HDL-C	LDL-C
---------	----	----	-------	-------

status	Mean ±SD	Mean ± SD	Mean ±SD	Mean ± SD
Normal range	70-150 mg/dl	150-240 mg/dl	30-70 mg/dl	70-130 mg/dl
Euthyroid	92.6 ^a ±48.91	172.7 ^a ±31	52.1 ^a ±11.49	104 ^a ± 32.6
Hypothyroid	189.2 ^{b**} ±106.2	227.6 ^{c**} ± 59.88	51.3 ^a ±17.13	149.0 ^{b**} ± 55.87
Subclinical hypothyroid	160.2 ^{b*} ±8.84	194.5 ^{b*} ±40.51	52.2 ^a ±9.58	116.3 ^{a*} ± 41.26

** means the p value was significant between this group and control group at ($P < 0.001$).

* means the p value was significant between this group and control group at ($P < 0.05$).

- Means within the same column having different small letters were significantly different.

Fig (14) Levels of Triglycerides (mg/dl) of the euthyroid, hypothyroid and sub clinical hypothyroid subjects.

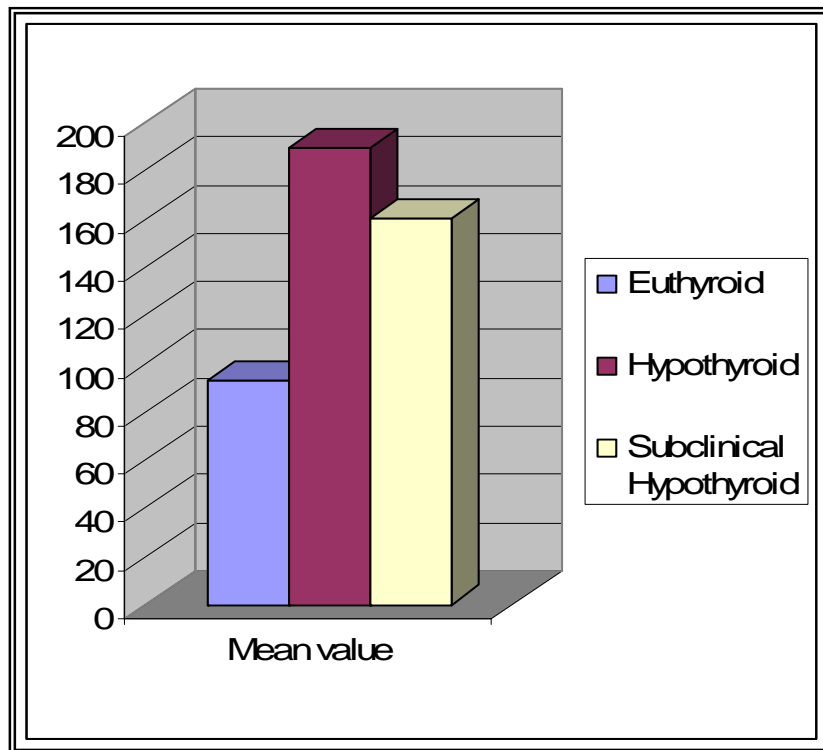


Fig (15): Prevalence of TG among euothyroid, hypothyroid and sub clinical Sudanese females under study.

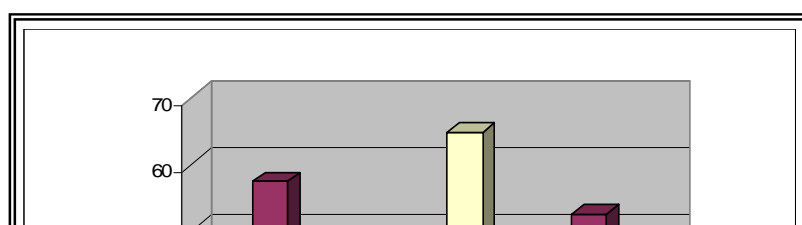


Fig (16): Mean values of Total cholesterol (mg/dl) of the euthyroid, hypothyroid and sub clinical hypothyroid subjects.

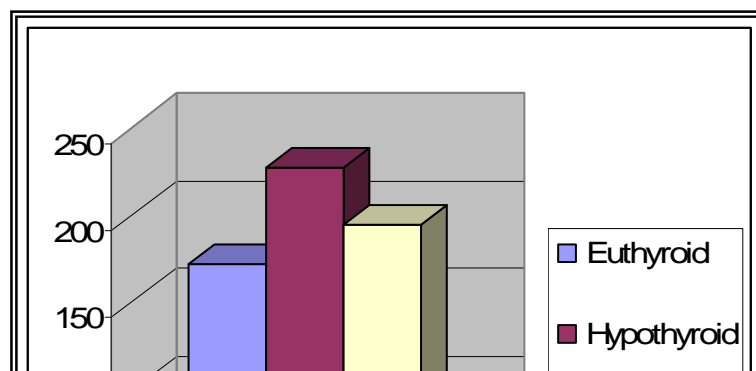


Fig (17): Prevalence of total cholesterol among subjects under study.

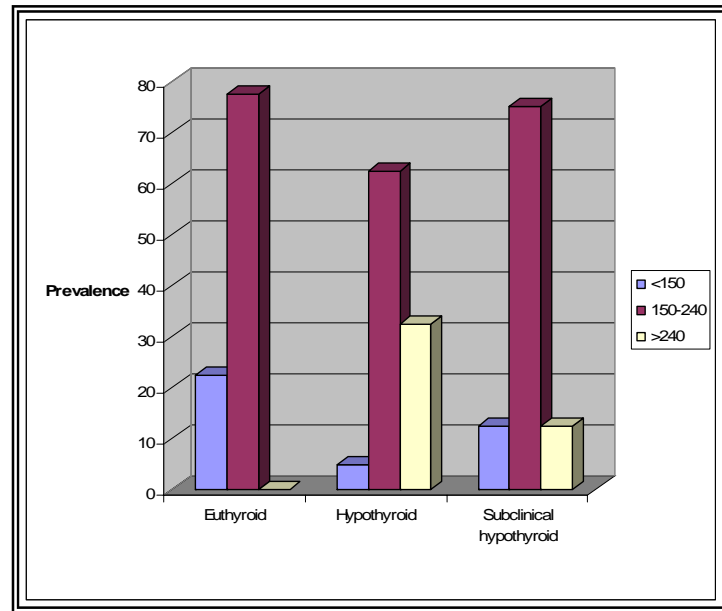


Fig (18): Values of HDL-Cholesterol (mg/dl) of the euthyroid, hypothyroid and sub clinical hypothyroid subjects.

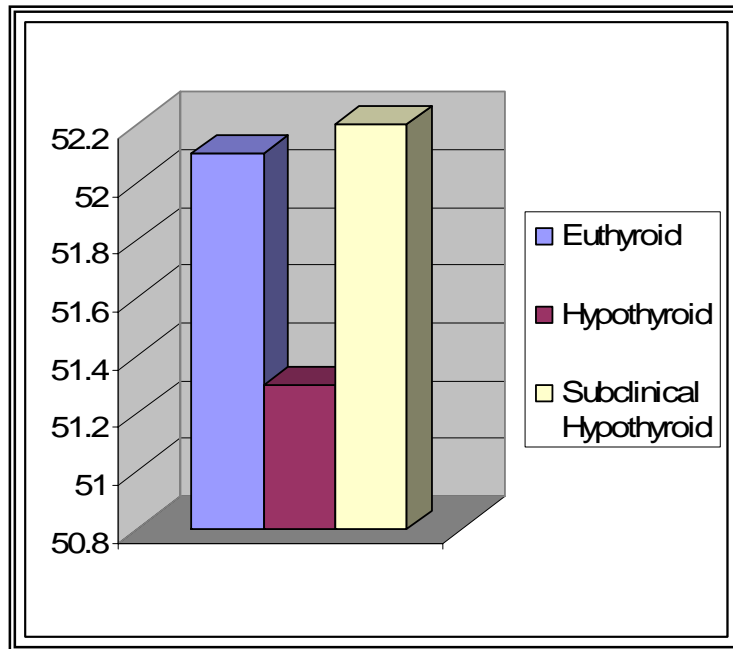


Fig (19): Prevalence of HDL-cholesterol among subjects under study.

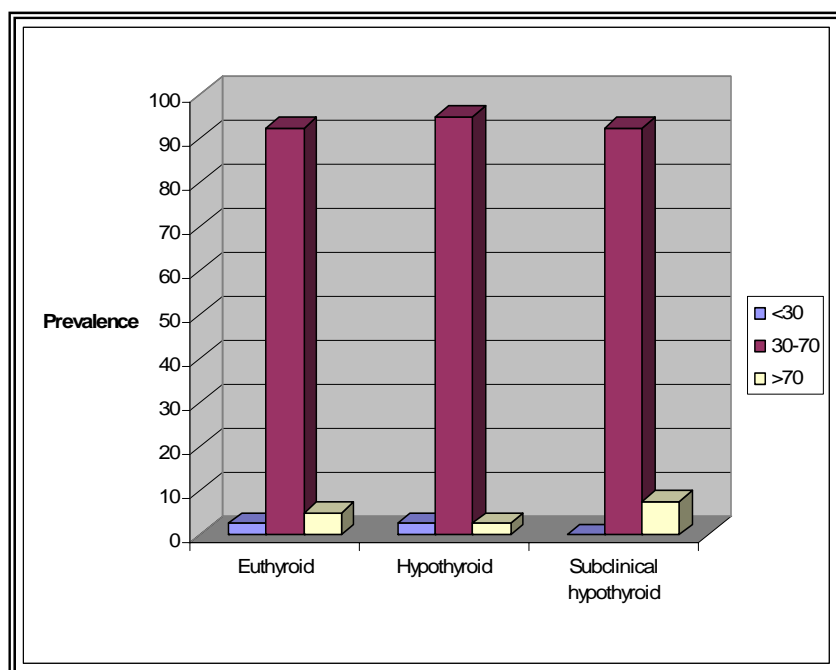


Fig (20): Values of LDL-Cholesterol (mg/dl) of the euthyroid, hypothyroid and sub clinical hypothyroid subjects.

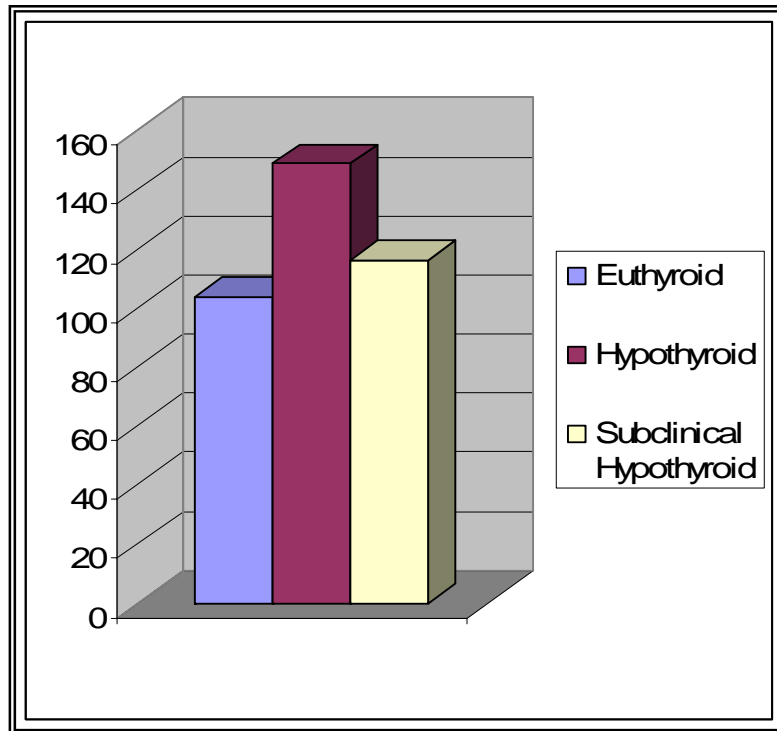
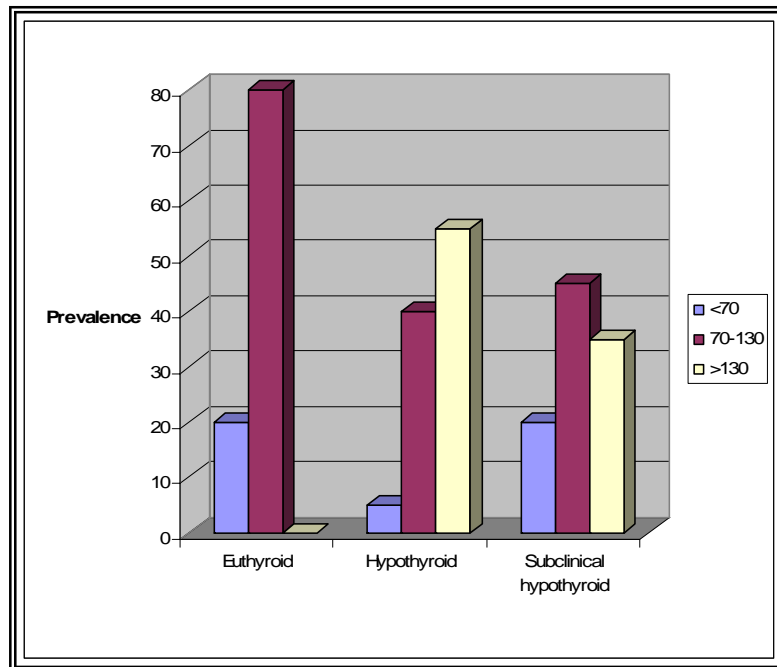


Fig (21): Prevalence of LDL-cholesterol among subjects under study.



Chapter Four

DISCUSSION

4.1 The Survey:

During the period from 1994 to 1996 about 15% of the Sudanese females referred to RIA laboratory of SAEC were found to be under treatment which is the same results found in males bearing in mind that 87% of the referred patients were females and this is in a good agreement with what has been published by Sukkar *et al.*, (2000) that all thyroid disturbances are more common in females than in males. 71.40% of the males were found to have euthyroid compared to 67.10% among the females who visited the laboratory in the same period. Those who had hypothyroid function among the males were found to represent 1.7% compared to 4.7% among the females. Sub- clinical hypothyroid was found to be 6.1% among the males and 6.2% among the females. To have a comprehensive study with regard to the study of the prevalence of these disorders; a detailed register should contain many parameters in addition to the data used to collect from the referred patients: that includes residence, tribe, family history of the disease or any endocrine disorder, treatment and any other informative data that can be used to reduce the risk factors and hence improve the quality of life for those who are considered to be under risk.

4.2 Thyroid status:

This study was designed to compare the level of some serum lipid parameters (cholesterol, TG, LDL- cholesterol and HDL- cholesterol) in hypothyroid and subclinical hypothyroid patients compared to euthyroid subjects. When T4 results were analyzed, the mean values were found to be 94.30, 18.4 and 89.7 nmole/L for euthyroid, hypothyroid and subclinical hypothyroid females respectively. While the mean values for T₃ were found to be 1.97, 1.19 and 1.95 for euthyroid, hypothyroid and subclinical hypothyroid females respectively. These findings were in agreement with levels reported by Series *et al.*, (1988), who reported low

plasma level of thyroxin (T_4) less than or equal to 45 nmol/L in four individuals evident of hypothyroidism, as apart of a screening program for coronary heart disease risk factors in the general population in Scotland.

In this study subjects that considered as sub clinical hypothyroid cases have obviously elevated TSH levels and normal values for T_3 and T_4 . Whereas these values were within normal range in euthyroid group and elevated TSH among hypothyroid groups. The mean values of TSH were found to be 1.60, 49.1 and 9.6 mIU/ml for euthyroid, hypothyroid and subclinical hypothyroid females respectively, go to table (6) page (44). These findings are explained by Adlin, (1998) who assumed that the elevation of TSH levels reflects the sensitivity of the hypothalamic-pituitary axis to small decreases in circulating thyroid hormone. As the secretion of the thyroid gland decreased, the TSH level may rise above the upper limit of normal range.

4.3 Serum lipids:

Triglycerides (TG) are the main storage lipid in adipose tissues and transported in the plasma via lipoproteins (Murray *et al.*, 2000). In this study the serum levels of triglycerides (TG) for different groups were as follows: among hypothyroid group, most cases showed high triglycerol levels, which reported mean level about 189.2 ± 106.2 mg/dl compared to control group that reported mean level about 92.6 ± 48.91 , however, there is significant variation between the euthyroid and hypothyroid females ($P < 0.001$). In previous studies the plasma TG were found to be elevated in hypothyroidism (Nikkila and Kekki, 1972). On the other hand, it was demonstrated that triglycerides metabolism is disturbed due to thyroid hypo function but to smaller extent than that of plasma cholesterol (Brook and Marshal, 2001), also these findings agree with many authors who

found this observation is not surprising as hypothyroidism is known to exert widespread effect on hepatic triglycerides assembly and secretion (Muller, 1984 and Barbagallo, 1995).

Among sub clinical hypothyroidism group slightly high TG level was found, whereas some cases remained within normal ranges, in comparison to euthyroid group that most of them were found within normal TG level, significant variation was found between euthyroid and subclinical hypothyroid ($P < 0.001$).

Total cholesterol levels among hypothyroid females were high with mean (227.6 ± 59.88), compared to the control group, which represented normal cholesterol level (172.7 ± 31). In case of sub clinical hypothyroid female cholesterol level was slightly elevated with mean about 194.5 ± 40.51 . Highly significant variation was observed between euthyroid and hypothyroid females for total cholesterol with ($P < 0.001$); the same result was found between euthyroid and subclinical hypothyroid females. Since the plasma cholesterol level is decreased by thyroid hormones, which increase the number of LDL receptors in the liver according to Ganong (2001), these results are agreed with other findings by Kinlaw (1995), who assumed that an increased risk for atherosclerosis in sub clinical hypothyroidism is thought to be due to the elevated cholesterol levels and the same results were found by Ball *et al.*, (1991) and Carantoni *et al.*, (1997).

Low-density lipoprotein (LDL) cholesterol fraction in this study was high in hypothyroidism and sub clinical hypothyroid females compared to euthyroid group which registered no elevation in these fractions, Highly significant variation was observed between euthyroid and hypothyroid females for LDL- cholesterol with ($P < 0.001$), the same finding was observed between euthyroid and sub clinical hypothyroid females. Study

done by Canaris *et al.*, (2000) noted significant elevation of LDL-cholesterol level in subjects with sub clinical hypothyroidism. The elevated LDL-cholesterol levels in hypothyroidism may occur as a result of increased cholesterol synthesis and absorption (Abrams and Grundy, 1981) decreased hepatic lipase and lipoprotein lipase activities (Valdemarsson and Nilsson-Ehle, 1982), and defects in the receptor-mediated catabolism of LDL (Chait *et al.*, 1979 and Thompson *et al.*, 1981).

Findings in the present study clearly showed that patients with hypothyroidism, their serum levels of triglycerides, total cholesterol and LDL- cholesterol are elevated, patients with sub clinical hypothyroidism, not surprisingly, the same changes are present but are less marked and less consistent. It is well known that hypothyroidism exhibits a reduction in the synthesis of cholesterol and in LDL catabolism, whereas cholesterol reabsorption is unchanged or even enhanced (Williams, 1997). The effect of sub clinical hypothyroidism on serum lipid levels and cardiovascular disease remain surrounded by controversy. However in the present study hypercholesterolemia was a common criterion among hypothyroid and sub clinical hypothyroid females.

The HDL-cholesterol levels were found to be within the normal ranges in all groups of this study, with no significant variation between the euthyroid and hypothyroid females and between the euthyroid and sub clinical hypothyroid females which means that there is no significant influence to the HDL-C levels. This also was found by Sasidharan, (1996).

This pattern of lipid abnormalities, of course, is important because it is a risk factor for atherosclerotic cardiovascular disease. Some studies done by Arem and Patsch, (1990); Franklin *et al.*, (1993) but not

others, (Cooper *et al.*,1983) have shown a decrease in LDL-cholesterol and total cholesterol levels after treatment with levothyroxine.

It is generally well known and repeatedly published that patients with hypothyroidism and sub clinical hypothyroidism show increased levels of cholesterol. This is believed to be resulted from impaired metabolism due to a decreased thyroid function (Williams, 1997). The same results are found to be true for LDL-C and TG among the hypothyroid and sub clinical hypothyroid subjects in the present work.

In some studies the relation between TSH and total cholesterol or LDL-C is inconsistent. Moreover, Williams and Williams, (2004), in a population-based study, concluded that sub clinical hypothyroidism does not appear to be associated with abnormalities in serum cholesterol or TG levels when adjusted for confounding variables. A more controversial issue is whether sub clinical hypothyroidism poses an increased risk for atherosclerosis. In summary, both hypothyroidism and sub clinical hypothyroidism are associated with increased levels of TG, cholesterol and LDL compared to that in the euthyroid state.

Conclusion

The present study is carried out to analyze the records of thyroid function test (TFT) statistically to know the average of thyroid gland

hypo function for subjects referred to RIA laboratory of SAEC (1994-1996) by using micro soft excel, and estimate the levels of some lipid profiles from blood samples of Sudanese females that are either suffering from clinical or sub clinical hypothyroid function.

Subjects with sub clinical hypothyroidism showed higher serum levels of cholesterol, LDL, and TG which are significantly different from the euthyroid subjects. Unfortunately, records on the levels of the thyroid hormones in euthyroid subjects and cases of thyroid dysfunctions are lacking in Sudan. Also there is no any kind of studies that focus on changes in the plasma lipids profile associated with sub clinical hypothyroidism.

Recommendations

- 1- To the Endocrinology laboratories:
 - a. To modify the registration form for the referred subjects to include tribe, original residence, family history of the disease, economical status.
 - b. To make sure that age, sex and treatment are fully addressed.
 - c. To make use of samples to conduct research in the biochemistry and physiology of human being after getting the permission.
- 2- To do lipid profile testing for patients who have clinical or sub clinical hypo thyroid functions.
- 3- A good filing system must be follow to facilitate the knowledge of the follow up of the thyroid disorders since Sudan was considered as endemic goiter area (Eltom *et al.*, 1984)
- 4- A network of researchers from the different laboratories should be connected to share the knowledge of the different disorders and research results.

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